Trace Nutrients Research 31 : 72－79 (2014)

Original Article

Increased Urinary Phosphate Excretion Induced by Fibroblast Growth Factor 23 in Zinc-deficient Rats

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Summary

Introduction: Zinc (Zn) is an essential trace element in cellular metabolism and skeletal mineralization. It is well known that 90% of Zn in the entire body is found in muscle and bone. Fibroblast growth factor 23 (FGF23) is a key regulator of Pi homeostasis and contributes to several hypophosphatemic disorders through decreased expression of the Na-Pi cotransporter type IIa (Na/Pi IIa) and Na/Pi IIc on the renal cortical brush border membrane. Whether FGF23 regulates Pi metabolism in Zn-deficient (ZnD) rats is unclear. Therefore, we measured the serum levels of FGF23 and 1,25-dihydroxy vitamin D_3 (1,25-VD_3) in ZnD rats, as well as the mRNA levels of bone FGF23, renal Klotho, Na/Pi IIa and Na/Pi IIc. Methods: The bone mineral density (BMD) of the femur and urinary Pi excretion estimated using a dual X-ray absorption method. Control groups consisted of rats on a paired calorie Zn-sufficient diet (Pf), and rats that were allowed to feed freely (F). Results: Compared with the Pf group, the serum levels of 1,25-VD_3 in the ZnD group were significantly reduced. Bone FGF23 mRNA expression, serum levels of FGF23, and urinary Pi excretion were significantly increased compared with the Pf group. The expression of Na/Pi IIc was significantly decreased compared with the F groups. Expression of Klotho mRNA was inhibited by Zn deficiency. Conclusion: These results suggest that Zn deficiency may inhibit 1,25-VD_3 synthase, resulting in increased serum FGF23. Moreover, increased FGF23 may reduce the reabsorption of Pi, resulting in a decrease in BMD.

Keywords: Fibroblast growth factor 23, Urinary phosphate excretion, Bone mineral density, Sodium/phosphate cotransporters, Zinc-deficient rat.

Abbreviations: Zn, zinc; Pi, phosphate; Ca, calcium; Cre, creatinine; 1,25-VD_3, 1,25-$(OH)_2$-vitamin D_3; ZnD, zinc-deficiency; BMD, bone mineral density; Na/Pi IIa, sodium/phosphate cotransporter type IIa; PTH, parathyroid hormone; FGF 23, fibroblast growth factor 23; FEP, fractional excretion of phosphate.

Introduction

Zinc (Zn) is an essential trace element that is an important nutrient cofactor of numerous enzymes[1)], transcription factors[2)], and an intracellular signaling mediator[3, 4)]. Physiological Zn is a cofactor of more than 300 enzymes, and more than 2,000 Zn-dependent proteins involved in stabilizing protein structures. Zn deficiency leads to anorexia, loss of appetite, failure of smell and taste, human growth retardation, and alopecia and osteoporosis in animals[5-8)]. It is important to realize that Zn deficiency is a major worldwide health problem with more than 30% of the global population at risk, predominantly in underdeveloped countries[9, 10)].

Of the Zn in the whole body, 90% is contained in muscle and bone[11)]. Bone is composed of inorganic mineral hydroxyapatite, which includes calcium (Ca), phosphate (Pi) and Zn. Metabolism of Ca and Pi in bone is strictly regulated by 1,25-$(OH)_2$-vitamin D_3 (1,25-VD_3) and parathyroid hormone (PTH). Increased PTH secreted from the parathyroid gland leads to an elevated serum Ca level, reduced serum Pi level, but increased urinary excretion of Pi, indicating that the balance of Ca and Pi represents an inverse relationship[12, 13)].

Shimada et al.[14)] cloned cDNA from a hemangiopericytoma that caused hypophosphatemic osteomalasia and

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found clones identical to fibroblast growth factor 23 (FGF23), which has been identified by positional cloning as a gene responsible for autosomal dominant hypophosphatemic rickets. They reported that FGF23 is secreted from osteocytes and osteoblasts. CHO-FGF23 tumors implanted into nude mice induced severe hypophosphatemia, growth retardation, rickets in the growth plates, deformities of the skeleton and reduced mineralization of bone. These bone disorders are related to Pi over-secretion from the kidney, suggesting an induced reduction of bone mineral density (BMD). Renal Pi excretion and reabsorption is mediated primarily by the sodium/Pi cotransporter type IIa (Na/Pi IIa) and Na/Pi IIc[15, 16]. FGF23 protein binds to the FGF receptor, bound by the transmembrane protein Klotho as a coreceptor[17, 18]. Klotho was discovered by Kuro-o et al.[19]. They indicated that the Klotho-knockout mouse induces aging. Another report indicated that PTH increases Pi excretion through FGF23 and Klotho[20]. Therefore, the regulation of Pi in Zn-deficient rat may be affected by FGF23 production.

In this study, the effect of Zn deficiency on FGF23, PTH, 1,25-VD^3, mRNA Klotho expression, Pi metabolism through Na/Pi IIa and Na/Pi IIc, and BMD were evaluated.

Materials and Methods

Chemicals

All chemicals were purchased from Wako Pure Chemical Industries (Osaka, Japan) or Sigma-Aldrich (St Louis, MO, USA).

Animals, and urine, serum and tissue sample preparations

All animal experiments were approved by the Animal Research Committee of Saitama Medical University (Approval number; 25M072). Pathogen-free, male, Sprague-Dawley rats (mean ± SD), 106 ± 0.8 g, n = 30) were purchased from Tokyo Laboratory Animal Science Co. (Tokyo, Japan). The rats were acclimatized in individual cages in a temperature-controlled room (22-24℃) with a 12-hour (h)/12-h light/dark cycle for 1 week. The rats were then divided into three groups and fed either a Zn-deficient diet (ZnD group, n = 10), a Zn-sufficient diet (5 mg/100 g Zn) that was calorie paired with the ZnD group (Pf group, n = 10), or *ad libitum* (F group, n = 10) for 4 weeks, as reported previously[21]. The amounts of Zn in the diet of Pf rats were determined the day before from the ZnD-fed-rat level. After 4 weeks, the sera, kidneys and femurs were obtained, and the samples were frozen in liquid nitrogen for subsequent real-time PCR analysis of FGF23, Klotho and Na/Pi II transporters. For evaluation of urinary excretion of bone minerals, the BMD level of the femur was also determined. The obtained sera were used for determinations of Zn, FGF23, 1,25-VD_3, PTH, Ca, Pi and creatinine (Cre).

Collection of urine samples and determinations of urinary Ca, Pi and Cre

To determine urinary secretion levels of Ca, Pi and Cre, urine samples were obtained using a KN-646 metabolic cage (Natsume Seisakusyo, Tokyo, Japan) over a course of 15 h (6 PM to 9 AM). The urine samples were stored at −80℃ until analyzed. The urinary concentrations of Ca, Pi and Cre were measured using a commercial kit (Ca for Calcium C-test, Pi for Phosphor C-test and Cre for Creatinine test; Wako Pure Chemical Inc., Osaka, Japan). The urinary levels of Pi and Ca were expressed as μg excreted Pi or Ca per 15 h per 100-g body weight.

Real-time PCR analysis

Real-time polymerase chain reaction (PCR) was performed using a Bio-Rad iQcycler (Bio-Rad Laboratories Inc., Hercules, CA, USA). For real-time PCR analysis of FGF23, Klotho, Na/Pi-IIa and Na/Pi-IIc, total RNA was obtained from the kidney and femur. Bone marrow was washed from the femur with saline, and the remaining thin bone was used for total RNA extraction. Total RNAs were extracted using ISOGEN (Nippon Gene, Tokyo, Japan), and cDNA was synthesized from the extracted total RNA using an iScript™ cDNA Synthesis kit (Bio-Rad Laboratories), following the manufacturer's protocol. The FGF23 primers (accession number; AB078777); Forward (F): 5'-ttggatcgtatcacttcagc-3', Reverse (R): 5'-tgcttcggtgacaggtag-3', the Klotho primers (AB017820), F: 5'-ggccgaccatttcagggattac-3', R: 5'-atcgggcagcagggatgaga3', Na/PiIIa primers (NM_013030); F: 5'-agcacctcgacatccatcat-3', R: 5'-cggagctcacctccaaca-3', Na/Pi IIc primers (NM_139338); F: 5'-agctgaagaatactgaccaactca-3', R: 5'-gaccacctggtgcagctt-3', and GAPDH (AF_106860); F: 5'-aaacccatcaccatcttcca3', R: 5'-gtggttcacacccatcacaa-3' were used for PCR. The synthesized cDNA, the specific primers and iQ™SYBR ® Green Supermix (Bio-Rad Laboratories) were used for PCR, using the manufacturer's recommended conditions (94℃ or 30 s, 60℃ for 30 s, and 72℃ for 60 s; 40 cycles). The results of the PCR amplification were compared with those of the GAPDH, and data were presented by $2^{\Delta\Delta CT}$values as relative fold-expression levels.

Determination of serum FGF23, PTH, and 1,25-VD_3

Serum levels of FGF23, PTH and 1,25-VD_3 were determined for each group at 4 weeks after implementation of the diet. The detection limits of the assay were 1 pg/ml PTH (electrochemiluminescence immunoassay, ECLIA), 5 pg/ml 1,25-VD_3 (radioimmunoassay with two antibodies), and 3 pg/ml FGF23 (ELISA), as reported previously[20].

Determinations of serum Pi, Ca, and Cre

To determine the serum levels of Ca, Pi and Cre, sera were obtained at 4 weeks after implementation of the diets. Blood samples were obtained from the abdominal aorta under deep ether anesthesia. The samples were then centrifuged at 3,000 rpm for 10 min, and the separated sera were used for determination of serum Ca, Pi and Cre. The concentrations of Pi, Ca and Cre in serum samples were determined with commercial kits (Wako Pure Chemical) using an Ultramark Micro Imaging System (Bio-Rad) microplate reader. The serum levels of Ca and Pi were expressed as mg/dl.

The fractional excretion of Pi (FEP) was calculated as:

FEP = (urinary [Pi] × serum [Cre]) / (urinary [Cre] × serum [Pi]).

Determination of BMD in the femur

For determination of BMD, the femur was obtained under deep ether anesthesia. The soft connective tissues and muscle were removed, and then the BMD of the cleaned bone was measured by DXA using a Lunar PIXImus densitometer (GE Healthcare Co., CT, USA). Triplicate measurements were made from each of six independent samples from each group.

Statistical analysis

Statistical analyses were performed using the Kaleida Graph 4.0 software (Hulinks Inc., Tokyo Japan). All data shown represent means ± standard error (SE) or standard deviation (SD). The statistical significance of differences among groups was calculated by one-way ANOVA followed by Tukey's test as a post hoc test. For all analyses, differences were considered to be significant at *p*-values < 0.05.

Results

Table 1 presents the serum Zn concentrations and body sizes of the rats prior to and at 4 weeks after implementation of the special diets. The serum level of Zn in the ZnD group was significantly diminished at 4 weeks, compared with the Pf and F groups as shown in Table 1. Characteristic alopecia of Zn deficiency was observed in 50 % of the rats in the ZnD group (data not shown). There was no apparent diarrhea. From the rats' movements, the ZnD group also appeared to be depressed. These are characteristic symptoms of Zn deficiency, as reported previously[21]. These characteristic symptoms indicate that this rat model resulted in moderate Zn deficiency.

The relative fold levels (mean ± SE as $2^{\Delta\Delta CT}$ values) of bone FGF23 mRNA were 1.51 ± 0.17 in the F group, 1.24 ± 0.18 in the Pf group and 2.85 ± 0.37 in the ZnD group compared to GAPDH, as shown in Fig. 1A. The FGF23 level in the ZnD group increased significantly compared to the Pf group ($p = 0.0001$), and ZnD group vs. F group ($p = 0.001$).

The serum levels of FGF23 in the ZnD group increased significantly, compared with the Pf group. The serum levels of PTH in the ZnD group were increased tendency ($p = 0.0467$ from the F group, $p = 0.093$ from Pf group). The serum level of 1,25-VD_3 in the ZnD group was decreased compared the Pf group ($p = 0.046$) as shown in Table 2.

There was no significant difference in the levels of serum Ca and Pi among the ZnD, Pf and F groups. In serum, the Ca concentration was 9.3 ± 0.8 mg/dl in the F group, 9.1 ± 0.7 mg/dl in the Pf group and 9.0 ± 0.8 mg/dl in the ZnD group, which were not significantly different. The serum levels of Pi were 7.2 ± 0.51 mg/dl in the F group, 6.5 ± 0.48 mg/dl in the Pf group and 6.3 ± 0.50

Table 1 Rat body weight and serum zinc level

	Pre-experiment (n = 10, g)	After 4 weeks (n = 10, g)	Serum zinc (n = 10, μg/dl)
Free group	176.7 ± 1.3	355.7 ± 10.8	118.5 ± 2.4
Pf group	175.4 ± 2.3	221.8 ± 1.6 a	107.3 ± 2.6
ZnD group	182.0 ± 1.6	180.8 ± 2.0 a, b	29.8 ± 4.0 a, b

Changes in rat body weight and serum Zn concentration. The groups had either free access to food (F), a paired-calorie Zn-sufficient diet (Pf), or a Zn-deficient diet (ZnD). The data are shown as means ± SE. The symbols (a) and (b) represent a significant difference ($p < 0.01$) between the ZnD group and the F and Pf groups, respectively.

Table 2 Serum FGF-23, PTH, and 1,25-Vitamin D_3

	FGF-23 (n = 8, pg/ml)	PTH (n = 8, pg/ml)	1,25-VD_3 (n = 8, pg/ml)
Free group	240.0 ± 9.8	6.05 ± 0.39	177.5 ± 27.5
Pf group	230.4 ± 12.8	6.23 ± 0.29	230.0 ± 11.5b
ZnD group	311.9 ± 20.1 a ,b	7.35 ± 0.38 a	182.2 ± 8.1a

Serum levels of fibroblast growth factor 23 (FGF23), parathyroid hormone (PTH) and 1,25-dihydroxy vitamin D_3 (1,25-VD_3). The groups had either free access to food (F), a paired-calorie Zn-sufficient diet (Pf), or a Zn-deficient diet (ZnD). The data are shown as means ± SE. The symbols (a) and (b) represent a significant difference ($p < 0.05$) between the ZnD group and the F and Pf groups, respectively.

mg/dl in the ZnD group, which were not significantly different.

The urine volume (ml/15 h/100 g body weight) was 1.44 ± 0.20 in the F group, 1.01 ± 0.24 in the Pf group and 1.67 ± 0.41 in the ZnD group in Fig. 2A. The urine volume level in the ZnD group decreased significantly compared with the Pf group ($p = 0.0085$). The level of excreted urinary Ca (μg/15 h/100 g body weight) was 61.6 ± 36.4 in the F group, 73.8 ± 85.4 in the Pf group and 25.2 ± 18.7 in the ZnD group. The level of excreted urinary Ca in the ZnD group was not significantly different compared with the Pf group.

The level of excreted urinary Pi (μg/15 h/100 g body weight) was 231.3 ± 71.9 in the F group, 3.3 ± 1.2 in the Pf group and 48.7 ± 11.6 in the ZnD group in Fig. 2B. The level of excreted urinary Pi in the ZnD group and Pf group was reduced significantly relative to the F group ($p = 0.0063$ and $p = 0.0014$, respectively), but the excreted Pi in ZnD group increased from the Pf group, which was not significantly different. The increased Pi reabsorption in Pf group compared with F group is physiological response, becaused the daily fed diet level of Pf group as same level of ZnD group is redued (about 50-60% of F group) from F group fed level.

The FEP level in Pf group was significantly reduced from the F group ($p = 0.0021$) and the FEP level in ZnD group was increased significantly ($p = 0.0093$) from the Pf group (Fig. 4A).

Although there was no significant difference among the ZnD, Pf, and F groups, the relative fold increases in renal Na/Pi IIa mRNA expression were 1.32 ± 0.04 in the F group, 1.52 ± 0.19 in the Pf group and 1.80 ± 0.11 in the ZnD group, which were also not significantly different (Fig. 3A).

The relative fold increase in renal Na/Pi IIc mRNA expression was 2.17 ± 0.17 in the F group, 1.63 ± 0.09 in the Pf group and 1.41 ± 0.13 in the ZnD group; the Na/Pi IIc level in the ZnD group was significantly different from that in the F group (0.0066), but not the Pf group (Fig. 3B).

The BMD level of femurs in the ZnD group was significantly lower than in the Pf ($p = 0.0032$) and in the F ($p =$

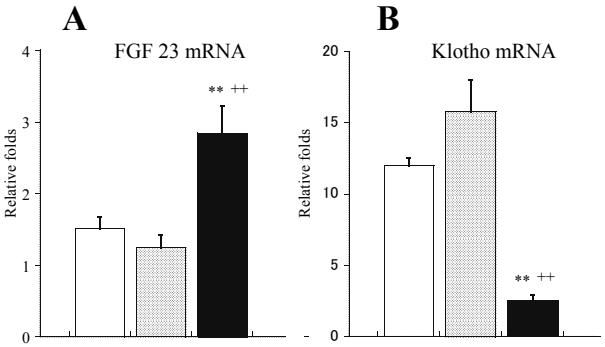

Fig. 1 A; Bone fibroblast growth factor 23 (FGF23) mRNA levels in rats as a function of diet, as determined by real-time PCR analysis. The groups had either free access to food (F, n = 5), a paired-calorie Zn-sufficient diet (Pf, n = 6), or a Zn-deficient diet (ZnD, n = 7); these groups are represented by an open bar, hatched bar or closed bar, respectively. The data are shown as means ± SE; ** and + + indicate a significant difference ($p < 0.01$) between the ZnD and Pf groups, and F group, respectively. B; Renal Klotho mRNA levels in rats. The groups had either free access to food (F, n = 5, open bars), a paired-calorie Zn-sufficient diet (Pf, n = 5, hatched bars), or a Zn-deficient diet (ZnD, n = 5, closed bars). The data are shown as means ± SE; ** and ++ indicate a significant difference ($p < 0.01$) between the ZnD and Pf groups, respectively, and the F group.

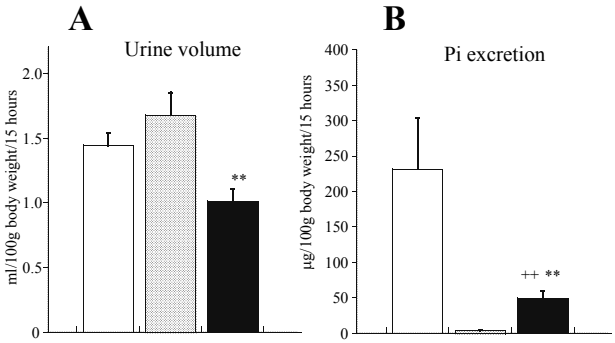

Fig. 2 A; Excreted urine volumes (ml/100 g body weight/15 h) in rats are shown. The urine samples were collected in a metabolic cage over a course of 15 h. The groups had either free access to food (F, n = 5, open bars), a paired-calorie Zn-sufficient diet (Pf, n = 5, hatched bars), or a Zn-deficient diet (ZnD, n = 6, closed bars). The data are shown as means ± SE; ** indicate a significant difference ($p < 0.01$) between the ZnD group and Pf groups. B; Excreted urinary Pi levels in rats (mg/100 g body weight/15 h) are shown. The groups had either free access to food (F, n = 5, open bars), a paired-calorie Zn-sufficient diet (Pf, n = 6, hatched bars), or a Zn-deficient diet (ZnD, n = 7, closed bars). The data are shown as means ± SD; ** and ++ indicate a significant difference ($p < 0.01$) between the ZnD group and the F and Pf groups, respectively.

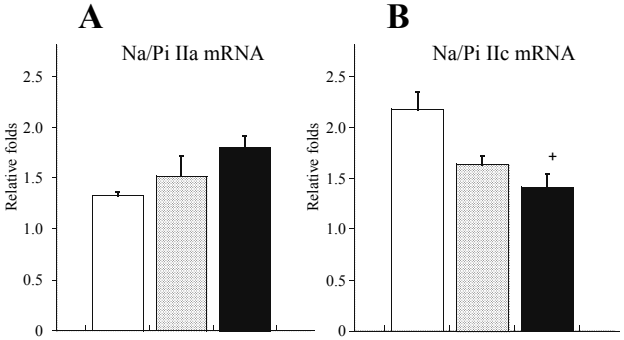

Fig. 3 Renal mRNA expression of Na/Pi IIa (A) and Na/Pi IIc (B) transporters. The groups had either free access to food (F, n = 5, open bars), a paired-calorie Zn-sufficient diet (Pf, n = 6, hatched bars) or a Zn-deficient diet (ZnD, n = 6, closed bars). The data are shown as means ± SE; + and * indicate a significant difference ($p < 0.05$) between the ZnD group and the F and Pf groups, respectively. Double symbols indicate $p < 0.01$.

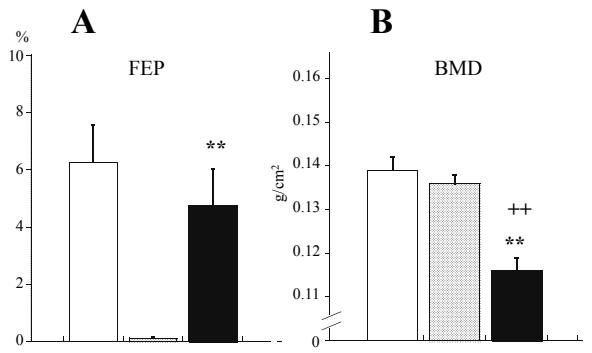

Fig. 4 A; The fractional excretion of Pi (FEP) in rats is shown. FEP values were calculated from the urinary and serum concentrations of Pi and creatine (Cre) according to:
FEP = (urinary[Pi] × serum [Cre]) / (urinary [Cre] × serum [Pi]).
The groups had either free access to food (F, n = 5, open bars), a paired-calorie Zn-sufficient diet (Pf, n = 6, hatched bars), or a Zn-deficient diet (ZnD, n = 6, closed bars). The data are shown as means ± SE; + indicates a significant difference ($p < 0.05$) between the Pf group and the F group; ** indicates a significant difference between the ZnD and Pf groups. B; Bone mineral density (BMD) of rat femurs, measured by the dual X-ray method. The groups had either free access to food (F, n = 7, open bars), a paired-calorie Zn-sufficient diet (Pf, n = 7, hatched bars), or a Zn-deficient diet (ZnD, n = 7, closed bars). The data are shown as means ± SE. Triplicate measurements were made from each of five independent samples from each group; + + and ** indicate a significant difference ($p < 0.01$) between the ZnD group and the F and Pf groups, respectively. Double symbols indicate $p < 0.01$.

0.0008) groups (Fig. 4B).

The relative fold increase in renal Klotho mRNA level was 12.0 ± 0.5 in the F group, 15.8 ± 2.2 in the Pf group and 2.5 ± 0.4 in the ZnD group in Fig. 1B. The renal Klotho mRNA level was significantly lower in the ZnD group than in the F ($p = 0.0040$) and Pf ($p < 0.0001$) groups.

Discussion

The serum level of the bone-derived hormone FGF23 and the FGF23 mRNA concentration in femurs increased significantly as a result of Zn deficiency in rats (Table 2). These increased FGF23 levels apparently inhibited the renal reabsorption of Pi, leading to increased urinary excreted Pi and reduced BMD of the femur in the Zn-deficient rats. These results suggest that Zn deficiency induces FGF23 mRNA expression, which would elevate the serum level of FGF23, resulting in a decreased BMD in the femur.

Serum levels of FGF23 are mostly derived from osteocytes and osteoblasts in bone, suggesting that the relationship between these cells and Zn ions may be important. Osteocytes secrete sclerosin[22], an inhibitor of bone formation, the phosphaturic factor FGF23[23], as well as other gene products, such as phosphate-regulating endopeptidase on the X-chromosome (Phex) that regulate bone mineralization and FGF23 expression[24-26]. The loss of Phex, an endopeptidase, by the *Phex gene* knockout model resulted in elevated circulating levels of FGF23 and inhibited renal Pi reabsorption in mice. Phex is a type I cell surface Zn metalloprotease, suggesting that Zn deficiency may reduce Phex activity. We speculate that the reduction of Phex inactivation by Zn deficiency may be related with increased serum FGF23 levels. Moreover, a Zn transporter, solute-carrier-39 (SLC39), belongs to a family whose members regulate Zn movement into the cytosol. Fukada et al.[27] indicated that SLC39 knockout mice induce defects in the maturation of osteoblasts, indicating that the depletion of intracellular Zn concentration in osteoblasts/osteocytes may involve FGF mRNA expression. It is likely that Zn deficiency also induces the depletion of systemic Zn levels in the serum and cytosol, resulting in an increase in serum FGF23. Hie et al.[28] reported that Zn deficiency in the rat reduced osteoblast differentiation and numbers through reductions in osteoblast specific transcription factor, rent-related transcriptional factor, Wnt signaling, and glycogen synthase kinase 3β. The depletion of these transcription factors by Zn deficiency may stimulate FGF23 transcription and elevate serum FGF levels.

Although the serum level of Pi was identical in both the ZnD and Pf groups, the level of excreted urinary Pi was greater in the ZnD group than in the Pf group. The Pi intake level from the diet and serum Pi level were identical in both the ZnD and Pf groups. Elevated FGF23 can stimulate urinary excretion of Pi through Na/Pi IIa and Na/Pi IIc[14-16]. In our study, the renal mRNA level of Na/Pi IIc in Zn-deficient rats was significantly different from those of the F group rat, which is consistent with the report by Hu et al.[16].

FGF23 is an important regulator of Pi homeostasis and vitamin D_3 metabolism. An increased circulating level of FGF23 results in hypophosphatemia because of renal Pi wasting and a low serum level of 1,25-VD_3[14]. In this study, the serum level of 1,25-VD_3 showed a tendency to decrease in Zn-deficient rats compared with the Pf group (Table 2). Furthermore, the activity of renal 1α-hydroxylase is reduced by Zn deficiency[29], which may result in reduced synthesis of 1,25-VD_3.

The serum Ca and Pi levels in ZnD rats were identical to those of Pf rats; nevertheless, the urinary excretion level of Ca in ZnD rats was lower than that in Pf rats (data not shown). Given that the balance of Pi and Ca is strictly controlled[13], increased renal excretion of Pi may induce

urinary Ca excretion. In addition, low 1,25-VD_3 reduces intestinal Ca absorption, presumably resulting in low Ca excretion.

Bone is maintained by osteoclasts and osteoblasts/osteocytes. BMD was reduced significantly in the Zn-deficient rats (Fig. 1B). Given that bone consists of Ca and Pi, the loss of Pi from the kidney is related to the loss of systemic Pi. Therefore, the reduced levels of BMD in Zn-deficient rat may be related to the increased urinary Pi excretion. A recent report[30] indicated that higher plasma FGF23 levels in elderly humans are correlated with reduced hip BMD and increased hip fracture risk. Moreover, Zn deficiency is often associated with diseases of the elderly, it indicates low BMD and osteoporosis; Zn supplements may improve these conditions[31-33].

The renal mRNA level of Klotho was reduced significantly by Zn deficiency, in agreement with a previous report[34]. Klotho-knockout mice display a phenotype almost identical to that of FGF null mice. Although Klotho is expressed abundantly in the kidney, the relationship between FGF23 and Klotho in kidney is unknown. Morishita et al.[35] reported that Zn addition (Zn orotate 0.25 g/100 g containing diet) in Klotho-knockout mice increased the survival rate and body weight compared to mice without Zn orotate. Although the exact mechanism is unclear, renal mRNA Klotho expression may be inhibited directly by Zn deficiency.

The Zn-deficient rats used in this study exhibited serum Zn depletion and growth retardation (Table 1), along with characteristic alopecia (data not shown), as reported previously[36]. These symptoms were reversed by a Zn-sufficient diet (5.0 mg/100 g diet) for 2 weeks (data not shown). We observed reduced urine volumes in Zn-deficient rats. Urine volume induced by Zn deficiency has been associated with renal blood flow[37], and daily water intake is affected by angiotensin II activity[38]. Indeed, angiotensin II centrally stimulates drinking behavior[39]. These factors may also be associated with water intake and urine excretion in Zn deficiency.

Hypozincemia is commonly found in patients with renal insufficiency or uremic patients on hemodialysis[39, 40]. These patients usually have hyperphosphatemia, resulting in the indirect induction of hyperparathyroidism. This increased level of PTH may have a secondary effect of reducing the serum 1,25-VD_3 level because Zn ions cannot stimulate osteoblastic proliferation through the Ca receptor[41]. Zn ions may not have a direct effect on PTH synthesis, release, metabolism, clearance and activity.

Conclusion

Therefore, Zn deficiency may inhibit 1,25-VD_3 synthase, resulting in elevated serum PTH, bone FGF23 mRNA, and serum FGF 23 levels. Furthermore, the increased serum FGF 23 level may reduce the reabsorption of Pi through Na/Pi IIa and Na/Pi IIc in the kidney, resulting in reduced femur BMD.

Acknowledgements

Thanks you to Takamasa Kido, (Department of Public Health & Environmental Medicine, The Jikei University School of Medicine) for his help with preparation of samples.

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